Estrogen-Glucocorticoid Interactions in the Uterus

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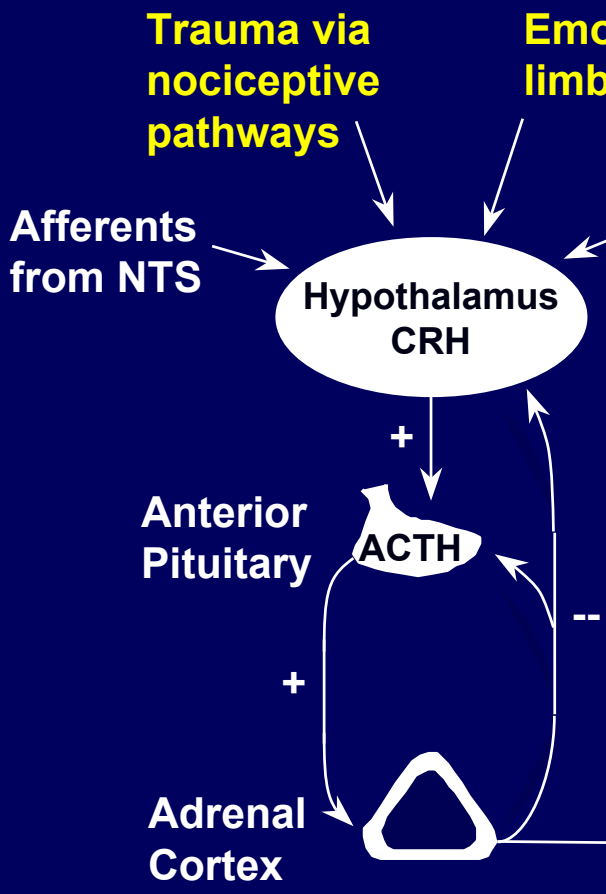
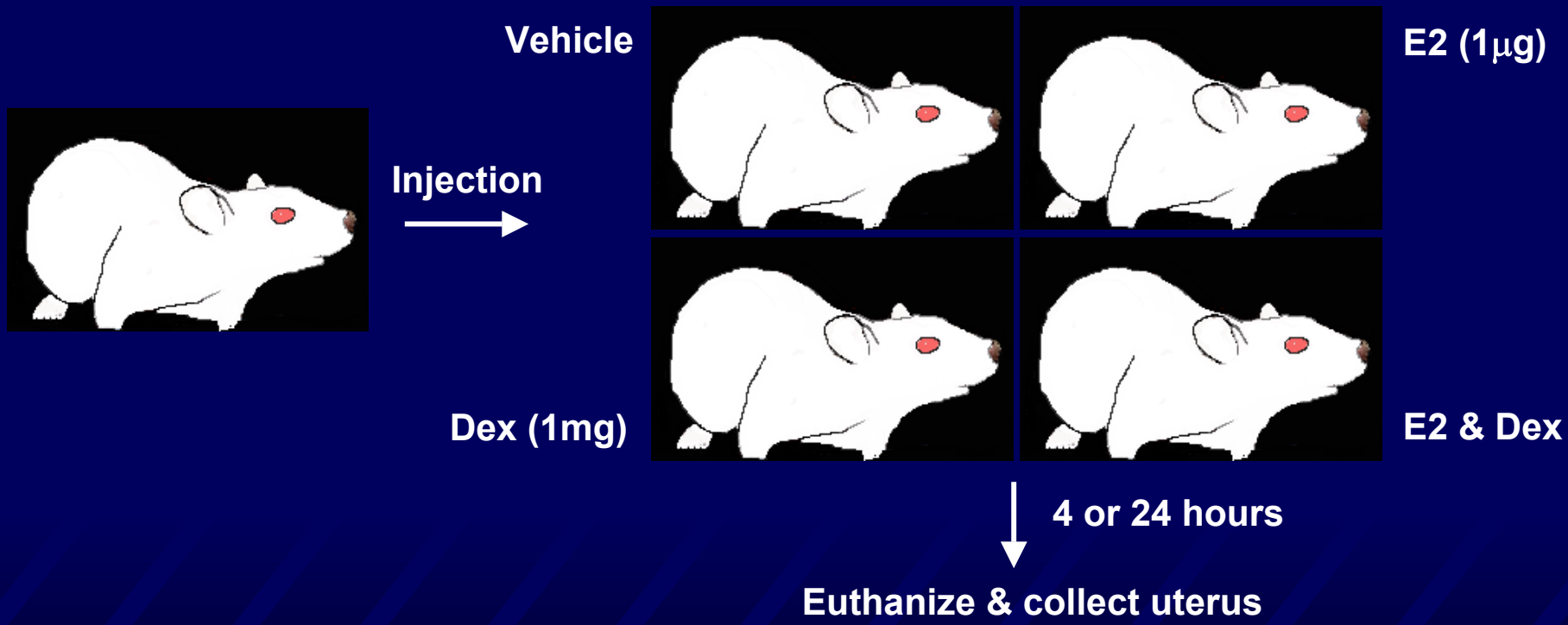


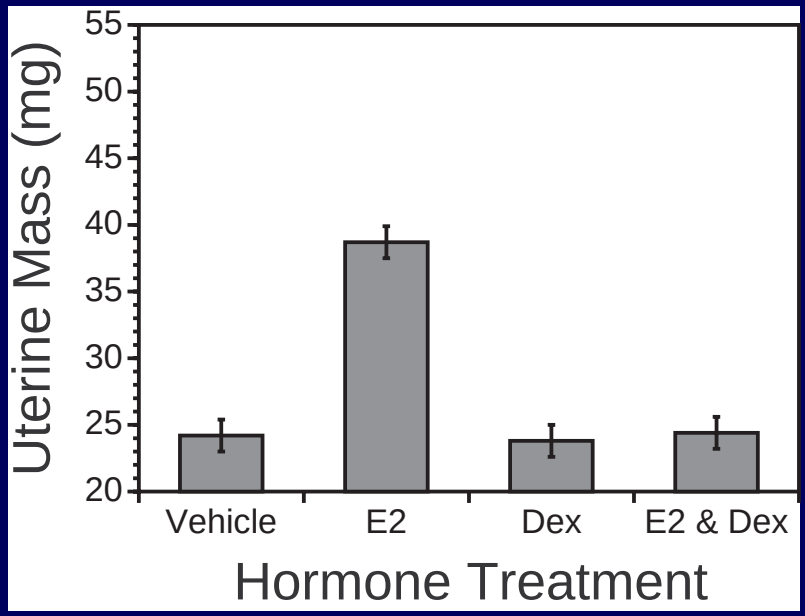
Glucocorticoids (cortisol, corticosterone)

Brain (behavior)
Liver (gluconeogenesis)
Muscle (protein degradation)
Fat (lipolysis)
Immune system (WBC apoptosis, anti-inflammatory)
Female reproductive tract (reproduction)

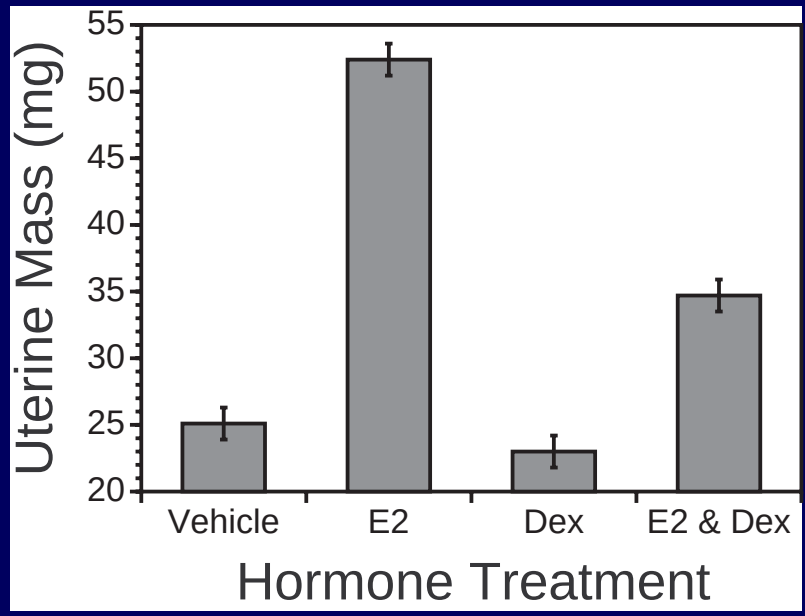
Model

- Study the effects of 17β-estradiol (E2) and dexamethasone (Dex) on uterine growth using immature female rats





4 Hours



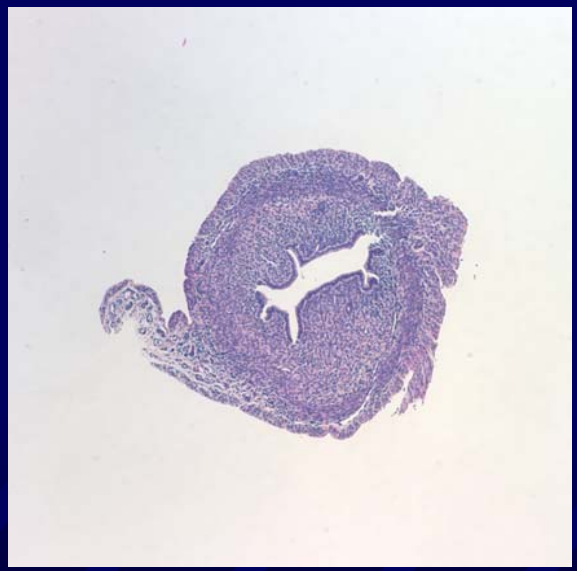
24 Hours

Cross sections of uteri 4 hours post-treatment

Vehicle

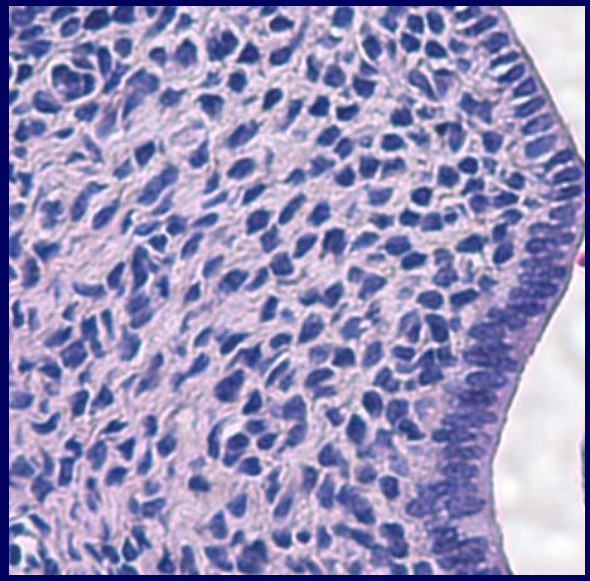
E2

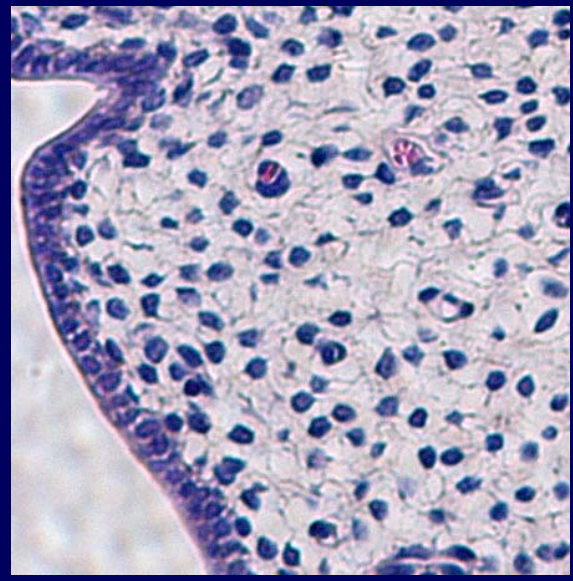
Dex



E2&Dex

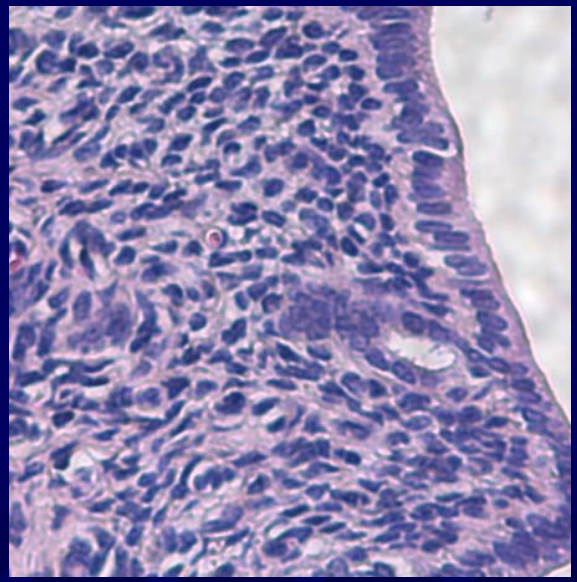
Vehicle

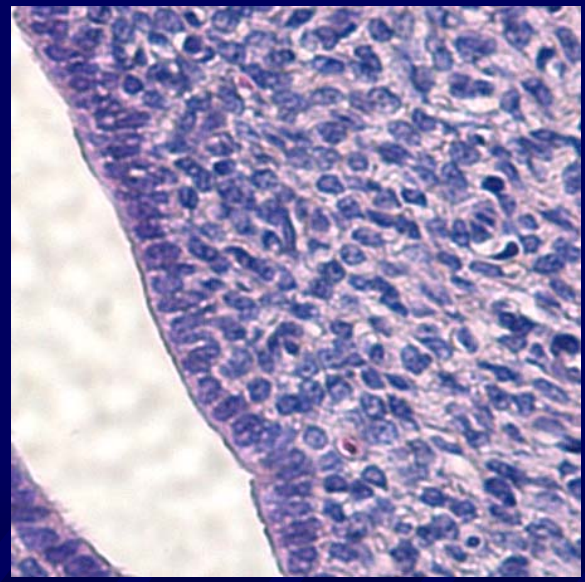




E2

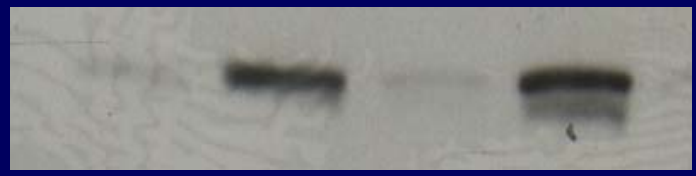
Dex



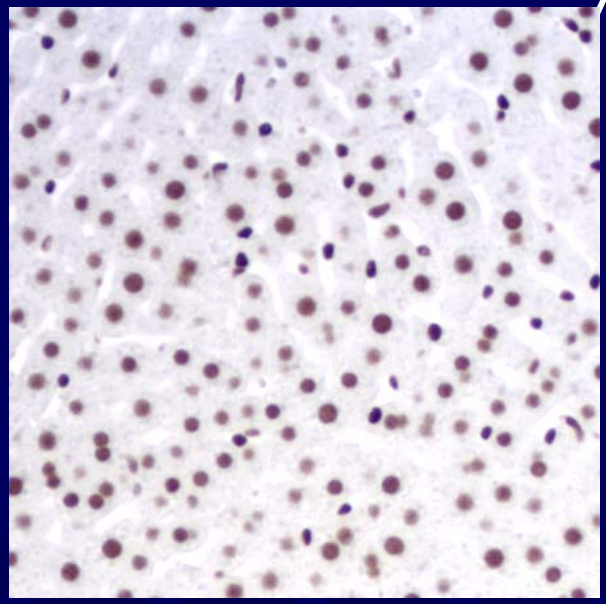


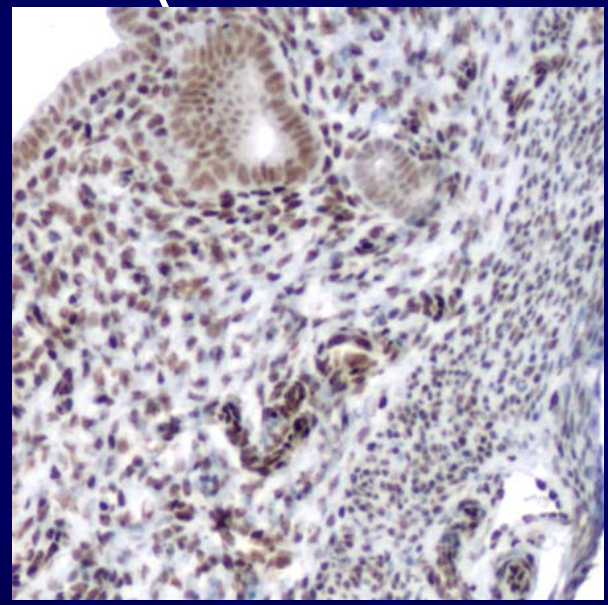
E2&Dex

GR is found at high levels in uterus

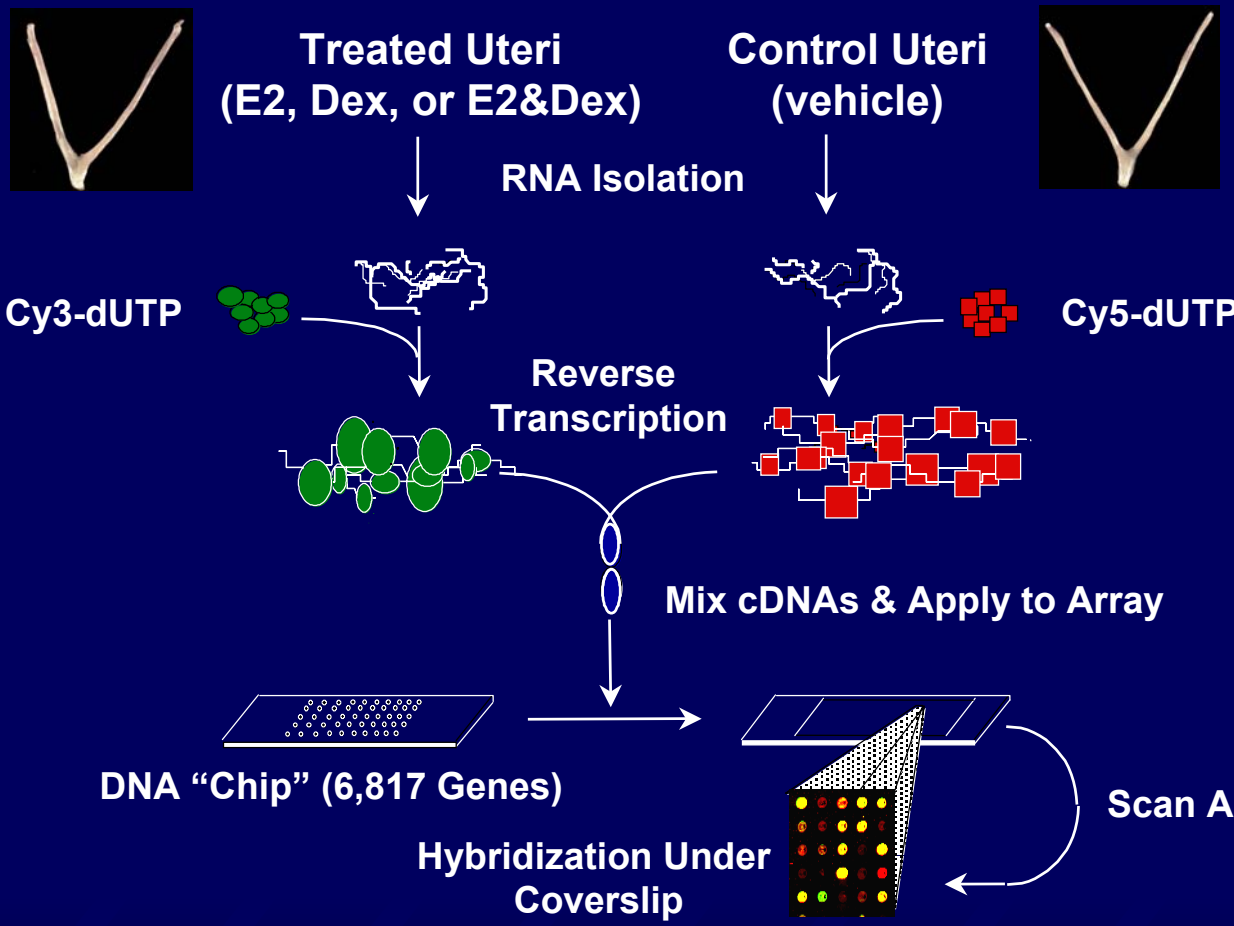


Brain Liver Muscle Uterus



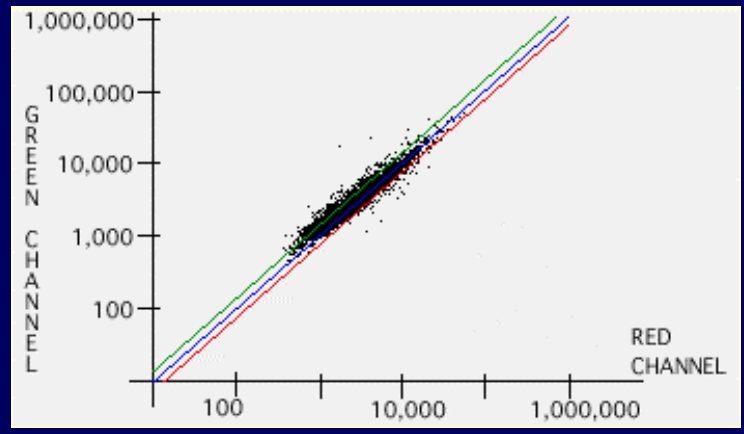


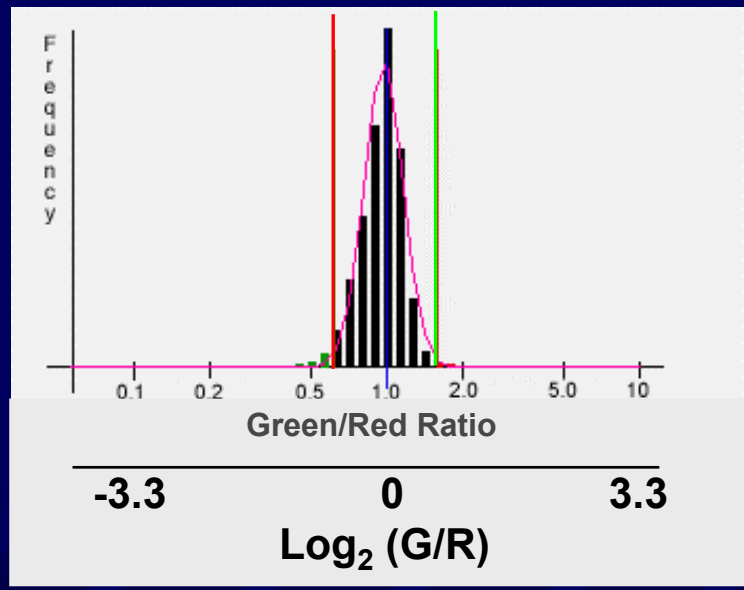
Microarray Procedure



Microarray Procedure

- Plot intensity for each gene in the green vs. the red channel
- Determine 95% confidence limits for the regression
- Genes falling outside these limits on 3/4 or 4/4 hybridizations are considered up- or down-regulated





General Results

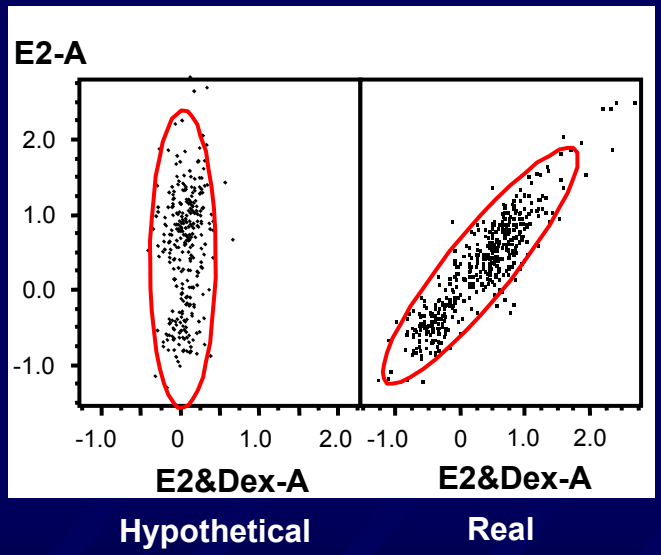
- 539 genes regulated by E2 and/or Dex (7.9 % of 6,817 genes)
- ~450 genes were regulated by E2
- ~240 genes were regulated by Dex
- Maximum G/R = 6.4 [log2 (G/R) = 2.68]
- Minimum G/R = 0.30 [log2 (G/R) = -1.74]

What are the patterns of gene expression induced by E2 and Dex?

- Principal components analysis
- Cluster analysis
- Functional analysis
- Individual genes

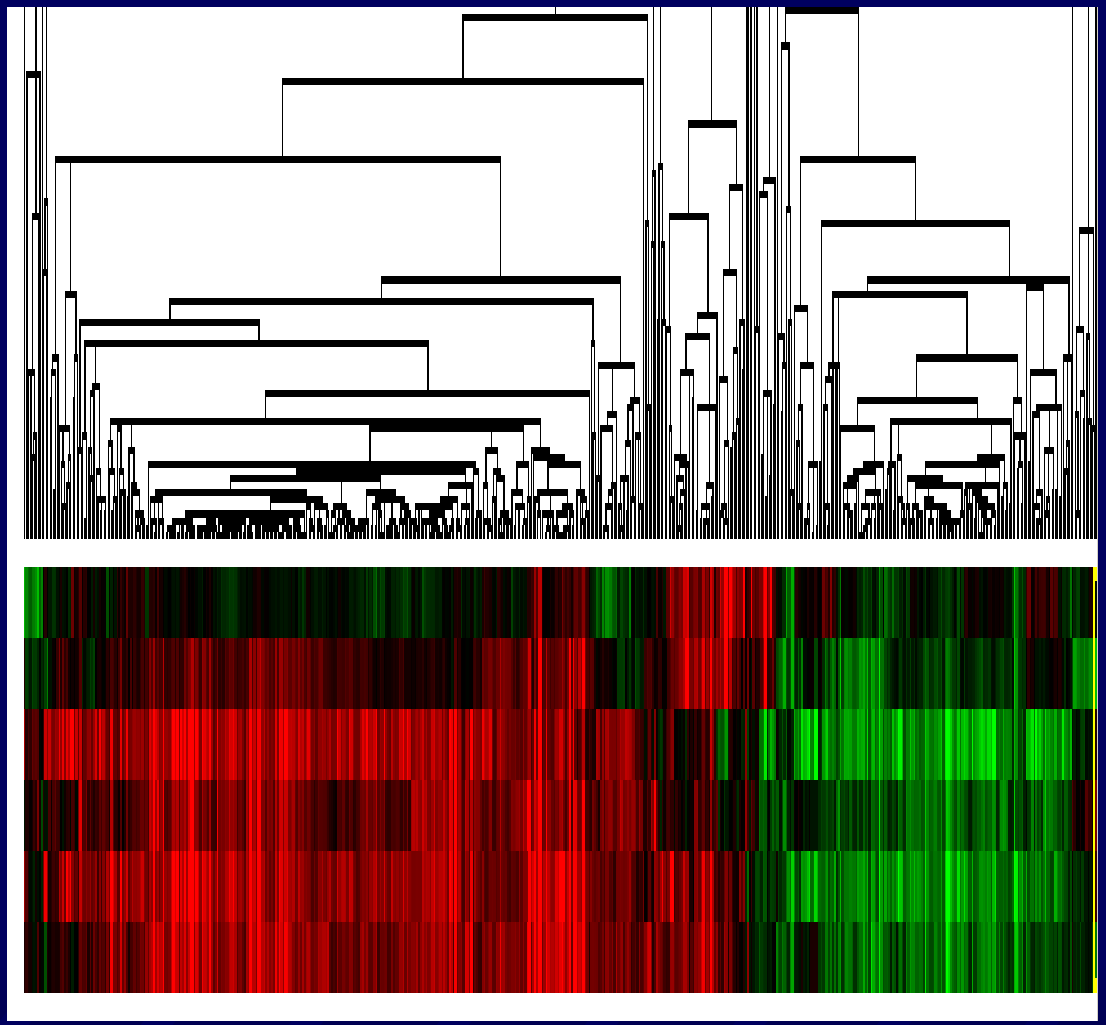
Principal Components Analysis

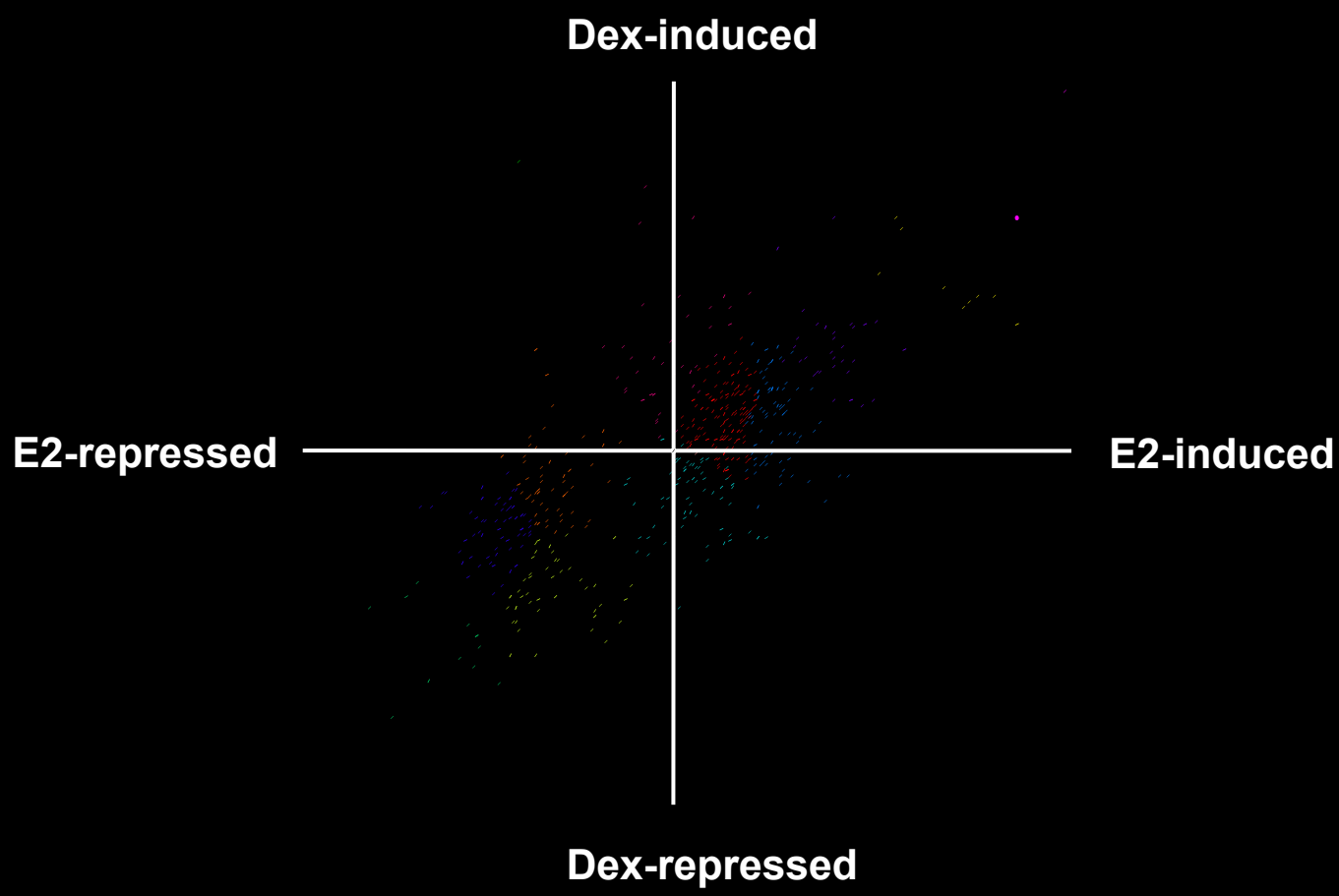
- Although Dex completely blocks the E2-induced increase in uterine mass and the histological changes seen at 4 hours, it has a disproportionately small effect on E2-regulated gene expression



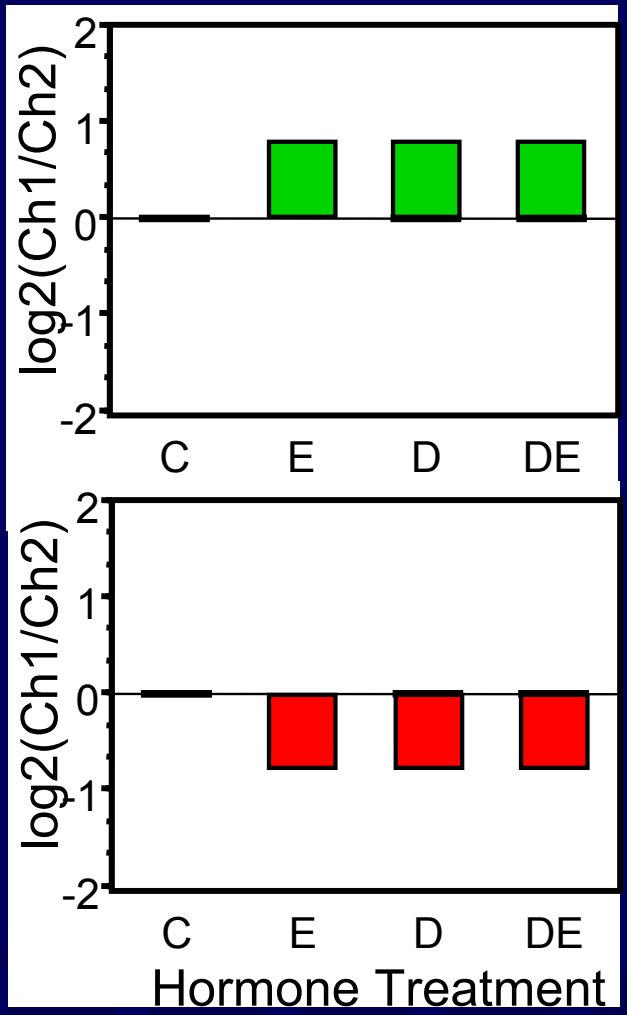
	E2 Effect	Exp. A vs. B	E2 vs. E2&Dex	Random variation
Eigen Value	3.72	0.13	0.11	0.04
Variation	93%	3.3%	2.7%	1%

Cluster Analysis




Dex-induced
E2-repressed
E2-induced
Dex-repressed

Cluster Analysis



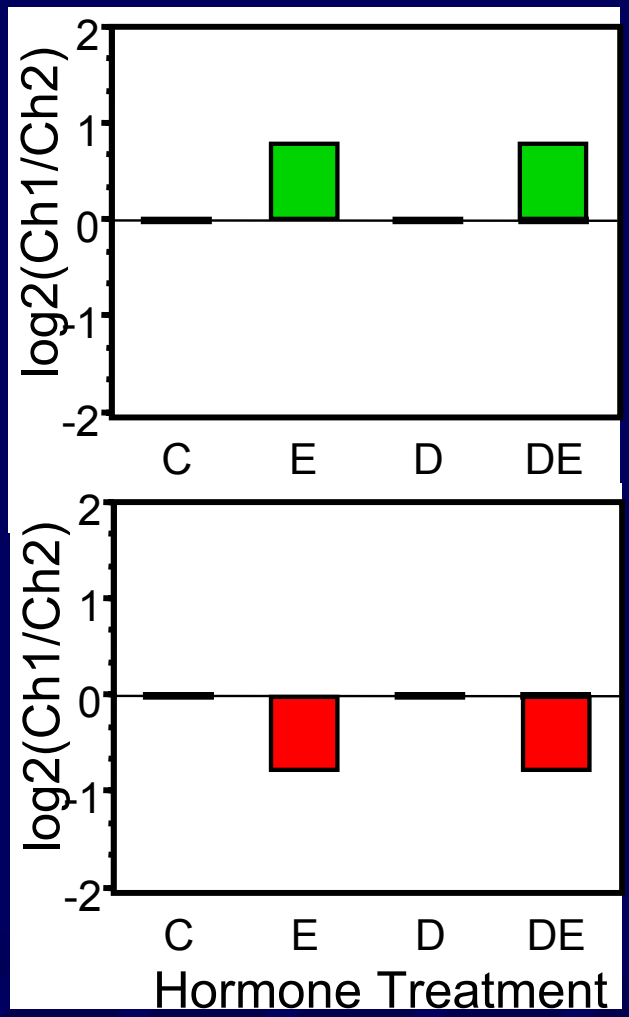
E2 and Dex-induced

- Galanin
- Metallothionein-1 and metallothionein-2
- Proteasome 26S subunit,ATPase (prosome,macropain)
- F-spondin

E2 and Dex-repressed

- Guanylate cyclase1, soluble, alpha 3
- Mindin precursor
- Lumican
- Coronin 1B (2)

Cluster Analysis



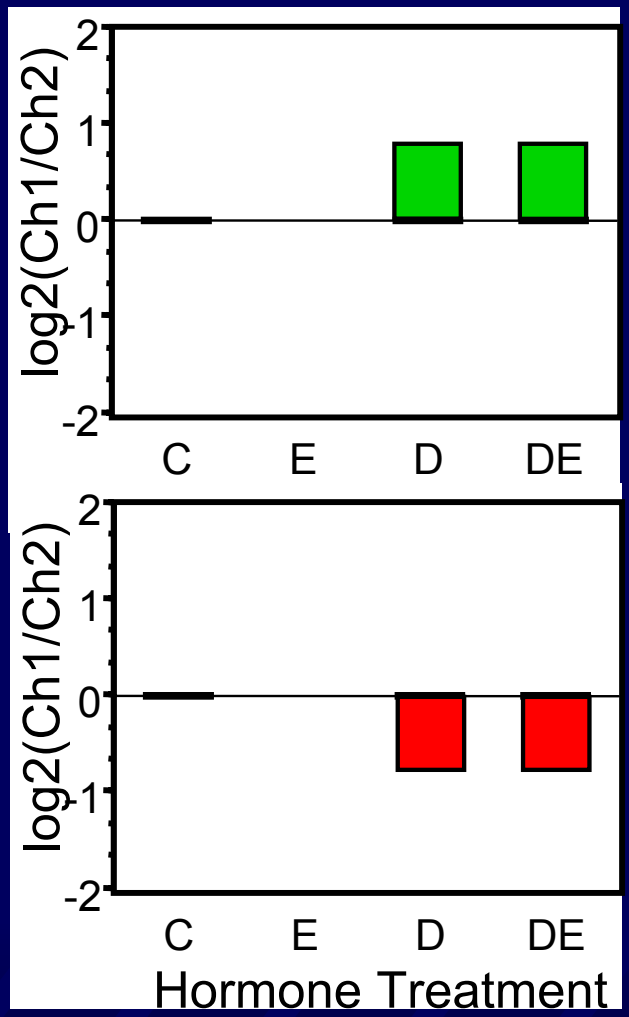
E2-induced

- Insulin-like growth factor-1
- Creatine kinase, brain
- Farnesyldiphosphate synthase
- Desmin
- Tissue inhibitor of metalloproteinase-1
- Hormone-sensitive-lipase,testicular-isoform

E2-repressed

- mRNA for Peptide/histidine-transporter
- Highly similar to Katanin-p80-Subunit [H.sapiens]
- Caspase 2
- Glutathione S transferase

Cluster Analysis



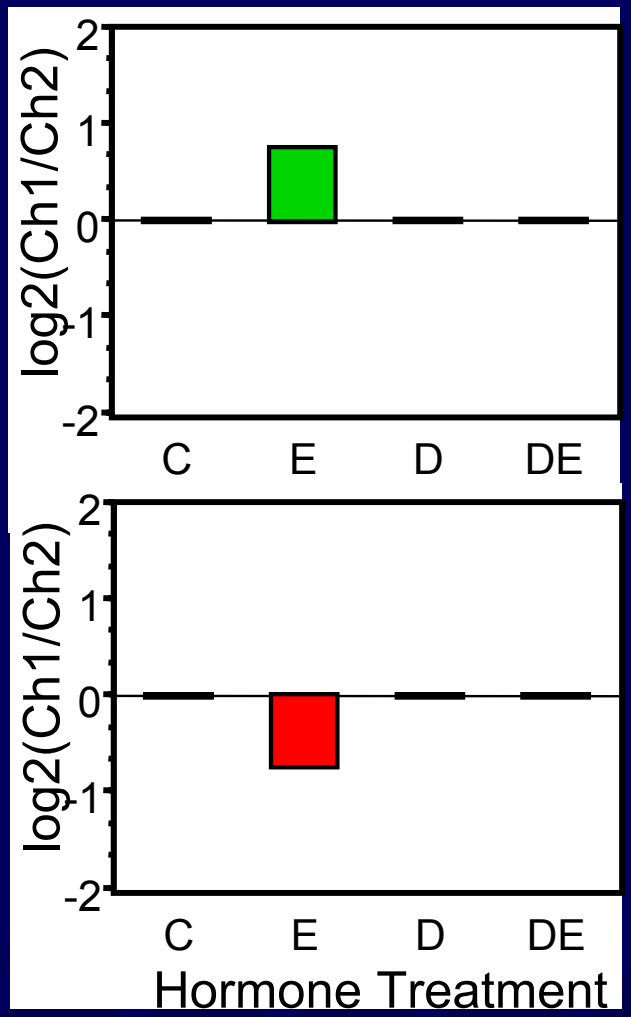
Dex-induced

AA955251 EST
AA819832 EST
AA955616 EST
AA955806 EST
Type-2A protein phosphatase, catalytic subunit mRNA

Dex-repressed

AA900495 EST
AA818796 EST
H2A histone family member *Z*

Cluster Analysis

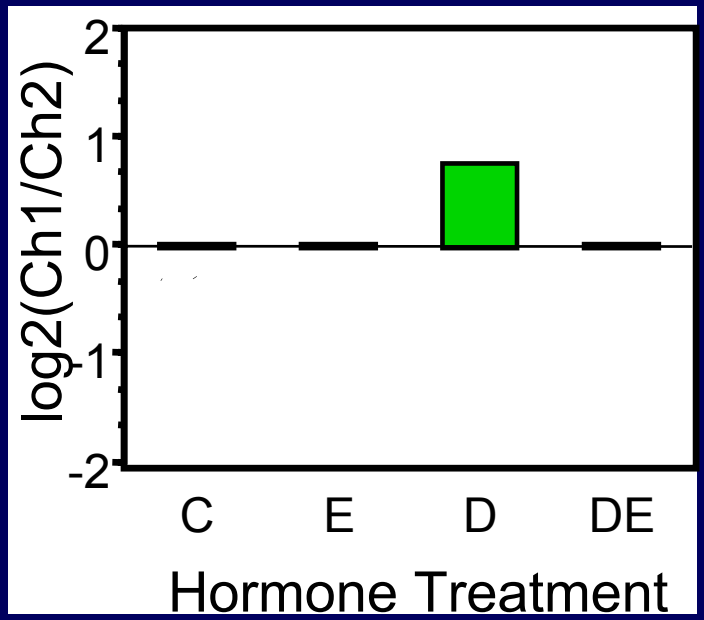


E2-induced (Dex blocked)

Rat pancreatic secretory trypsin inhibitor type II (PSTI-II) mRNA

E2-repressed (Dex blocked)

Cluster Analysis



Dex-induced (E2 blocked)

- Follistatin
- 11β-Hydroxysteroid-dehydrogenase
- Highly similar to A-Chain-A, murine collagen alpha 1 (Xv)Endostatin Domain

Functional Analysis (CS/ECM/Enzymes)

E2	Dex	E2&Dex
-	-	-

Lumican
EST MS to Rat Coronin 1B
Coronin 1B (actin binding protein)
Mindin precursor
EST HS to Microtubule Associated Protein EB1 (human
Adducin 3, Gamma
Extracellular Matrix Protein 2
Procollagen (type III, alpha 1)
Calpain-like Protease

E2 only	Dex	E2&Dex
-	0	-

Vimentin
EST Highly Similar to Katanin p80 Subunit (human)
Procollagen (type I, alpha 2)
EST Weakly Similar to Cathepsin H Precursor (rat)

E2	Dex	E2&Dex
-	++	+

Matrix Gla protein
EST HS to A-Chain, Collagen Alpha 1 (XV), Endostatin Domain (mouse)

E2	Dex	E2&Dex
+	0	0

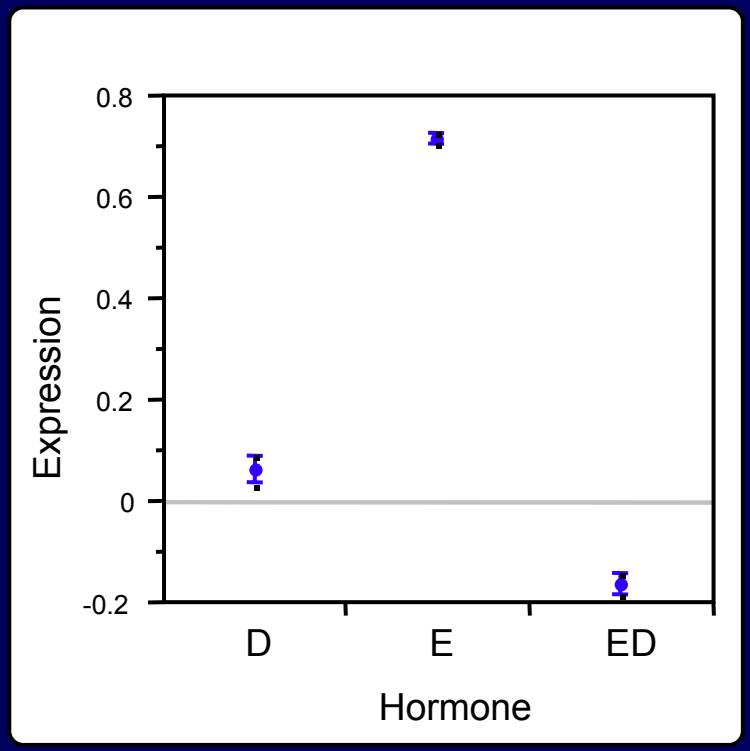
Pancreatic Secretory Trypsin Inhibitor Type II

E2	Dex	E2&Dex
+	0	+

Clathrin
Beta-tubulin 15
EST HS to S32604 Collagen (type VI, alpha 2) (mouse)
Tropomyosin 4
Cytoplasmic Beta-actin
Smooth Muscle Alpha-actin
Non-muscle Alpha-actinin 1
Supressor of Actin 1
Calponin
Integrin (alpha 6)
Integrin (beta 1)
Desmin
Cofilin 1, non-muscle
Ezrin
TIMP 1
Chymotrypsin B

Full Antagonism

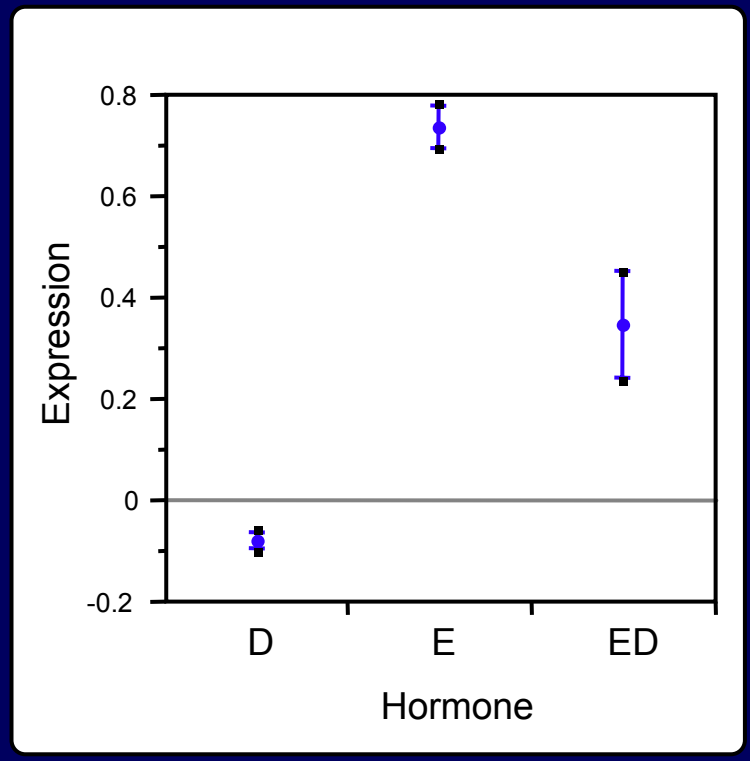
Pancreatic Secretory Trypsin Inhibitor, Type II



1 gene

Partial Antagonism

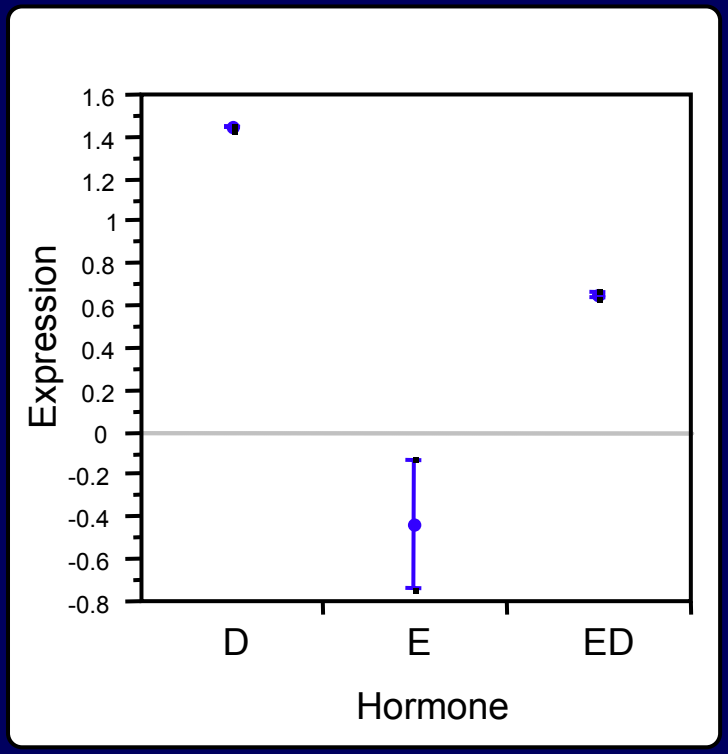
Iodothyronine Deiodinase, Type III



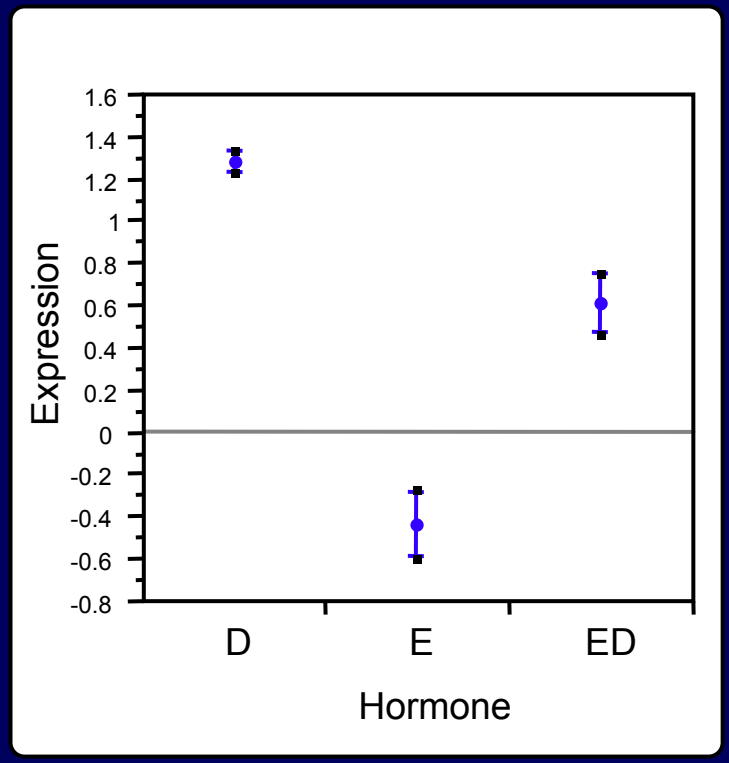
3 genes

Partial Antagonism

Alcohol Dehydrogenase, Class I



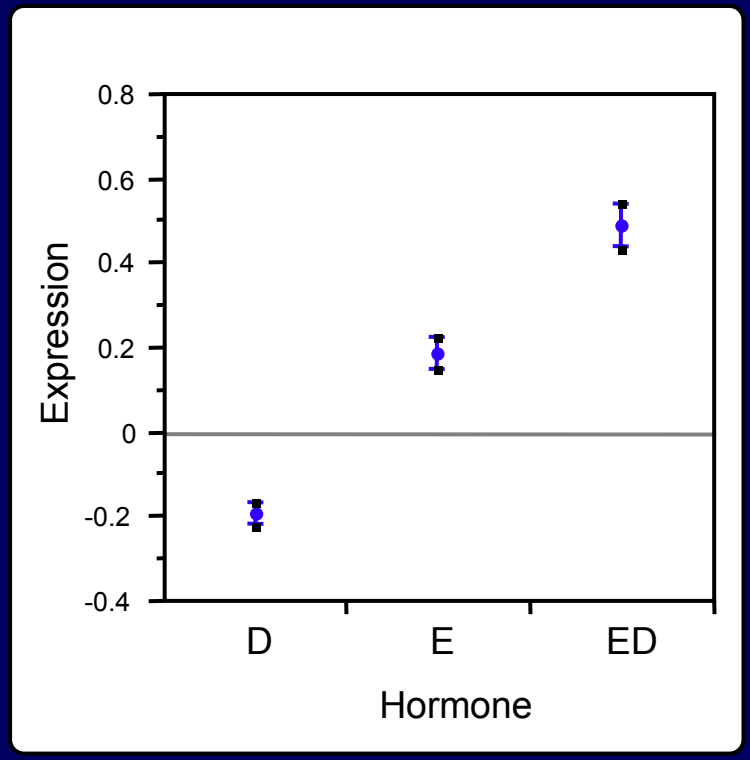
Matrix Gla protein



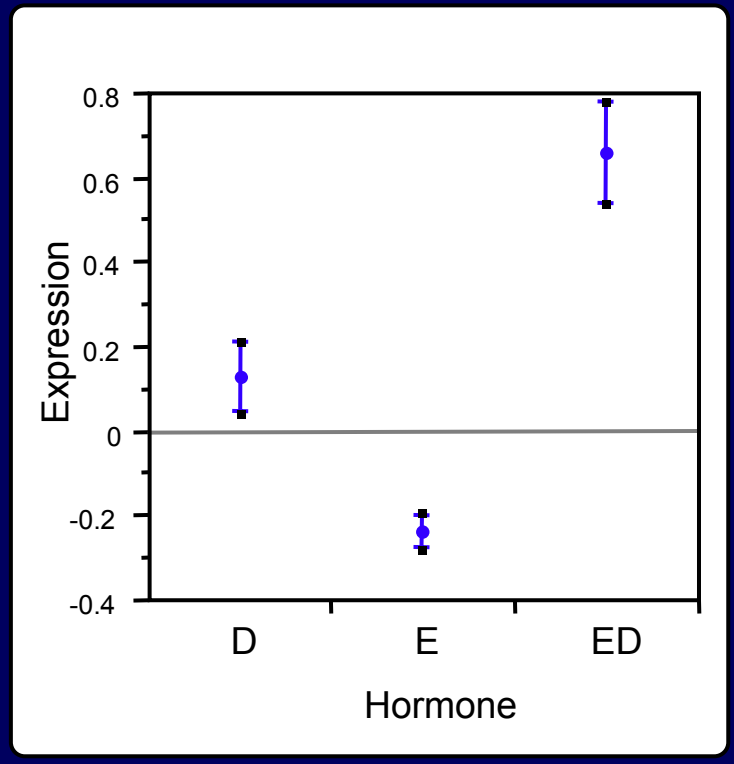
4 genes

Synergy

AA901170 EST



AA901294 EST



5 genes

Conclusions

- Many genes are regulated by E2 and/or Dex in the rat uterus
- However, Dex only antagonizes a small proportion of the genes regulated by E2
- There are diverse patterns of regulation, even for genes that have a common function (i.e., cytoskeletal & ECM proteins)

Future experiments

- Confirm regulation of representative genes
- Investigate transcriptional regulatory mechanism for E2 and Dex antagonism
  - Alcohol dehydrogenase, class 1 (retinol to RA)
  - Matrix Gla protein (prevention of ectopic calcification)
  - Pancreatic secretory trypsin inhibitor, type II

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